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IDENTIFICATION AND COMPARATIVE RISK ASSESSMENT OF AIRBORNE CARCINOGENS FROM COMBUSTION SOURCES

by

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The comparative mutagenicity and carcinogenicity of a series of combustion emissions has been assessed using dose-response studies in bacteria, mammalian cells and rodents. This data base has been used to develop a comparative potency risk assessment methodology for a series of combustion emissions.

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ABSTRACT

The identification and assessment of airborne carcinogens has been significantly advanced by the use of short-term genetic bioassays. Bioassay-directed fractionation coupled with new organic characterization methods has provided the tools needed to more efficiently identify potential carcinogens in complex combustion emissions and urban air samples. These studies have shown that a significant portion of the mutagenicity is found in fractions more polar than polynuclear aromatic hydrocarbons(PAHs). New mass spectrometry techniques have been developed to identify and quantify the more polar mutagens in these samples.

The comparative mutagenicity and carcinogenicity of a series of combustion emissions has been assessed using dose-response studies in bacteria, mammalian cells and rodents. This data base has been used to develop a comparative potency risk assessment methodology for a series of combustion emissions.

PART I: CHARACTERIZATION AND COMPARISON OF THE MUTAGENIC AND CARCINOGENIC ACTIVITY OF COMBUSTION EMISSIONS

INTRODUCTION

Incomplete combustion products from various sources contain carcinogenic polycyclic organic compounds and in several cases these combustion products have been recognized as human carcinogens (e.g., chimney soot, coal tars, and cigarette smoke). Polynuclear aromatic hydrocarbons (PAH's) and particularly benzo[a]pyrene (BaP) were implicated in the early 1900's as the major carcinogenic constituents of coal tars and soot extracts.[1-3] Since then, many PAH's have been isolated from combustion products, identified and evaluated as carcinogens in animals.[4-6] Although many other PAH's are carcinogenic, BaP was for many years considered the best indicator of PAH's and was monitored as a measure of both air pollution and occupational exposure to PAH's from combustion products.[5,7-9] Recent improvements in quantitative analytical methods for measuring many PAH's and other carcinogenic polycyclic organic matter (POM's) (e.g., aromatic amines, substituted PAH's, and heterocyclic compounds) have shown that BaP is not always well correlated with total PAH exposure or exposure to carcinogenic POM's.[10-12] Advances in bioassay-directed chemical fractionation and characterization techniques[13] when applied to different combustion products, also indicate that PAH's alone do not account for all the mutagenic or carcinogenic activity of these products.[14-16]

The relative mutagenic and carcinogenic activity of a series of these organic combustion emissions has recently been characterized and compared. The combustion emission sources include residential wood stoves and oil heaters,[17] utility power plants,[18] diesel and gasoline vehicles.[19] The relative

activity of these emissions has been compared to the activity of complex organics from roofing tar pots, coke ovens, and cigarette smoke; emissions for which human lung cancer data are available.[20-22] This chapter reviews these recent studies designed to: 1) use short-term mutagenicity assays to characterize fractionated combustion products, 2) evaluate the relative mutagenic and carcinogenic activity of combustion emissions, and 3) develop a comparative potency method for estimating cancer risk from combustion products.

CHARACTERIZATION OF THE MUTAGENS AND CARCINOGENS EMITTED FROM COMBUSTION SOURCES

Combustion of either fossil fuels (e.g., oil, coal, gasoline, diesel fuel) or vegetative sources (e.g., wood, plant materials) results in the production and usually the emission of a very complex mixture of organic gaseous and particulate pollutants as well as some inorganic materials. The major gaseous pollutants CO, NO_x, and SO_x are known to produce a variety of acute and chronic non-carcinogenic health effects associated with the respiratory system. These and other gaseous emissions such as nitrous acid (HONO), are thought to react with many of the organic emissions to form oxidized and nitrated compounds such as epoxides, lactones, and nitrated polycyclic aromatic hydrocarbons (NO_2-PAH's).[23] A number of these epoxides (e.g., BaP-4,5-oxide),[24] lactones (e.g., 4-oxapyrene-5-one)[23] and NO_2-PAH's (e.g., nitropyrenes)[25] have been shown to be mutagens and in some cases carcinogens. Combustion of both fossil and vegetative fuels also results in the emission of gaseous compounds which have been shown to induce tumors in animals or have been implicated as carcinogens in human epidemiological studies including aldehydes (e.g., formaldehyde, acetaldehyde) and aromatic hydrocarbons (e.g., benzene).[26,27]

Virtually every combustion process in which carbonaceous fuels are burned results in the production of soot. Soot often consists of clusters of very small (~30nm) spheroids fused together to form particles with a 0.1-0.2 micrometer diameter.[28] These soot particles contain an organic fraction (solvent extractable) and an elemental carbon fraction which appears to be similar to graphite.[28] The combustion of diesel fuel, gasoline, coal, fuel oil, and wood have all been shown to produce soot particles with extractable organic matter which is mutagenic in short-term bioassays[17-19,29-31] and in several of these sources have been shown to be tumorigenic in animals.[32-34] Chemical characterization of these organics shows that they contain carcinogenic PAH's. Recently, chemical characterization studies of motor-vehicle emissions have identified the presence of methylated PAH's (e.g., methylphenanthrenes),[35] nitrated PAH's (e.g., nitropyrene),[36] oxidized PAH's (e.g., cyclopenta(c,d)-pyrene dicarboxylic acid anhydride),[37] and a variety of other polycyclic organic compounds not yet evaluated in animal cancer bioassays.

Bioassay-directed fractionation closely coupled to chemical characterization has been shown to be the most efficient and effective approach to identifying the biologically active compounds in a complex mixture. This approach has been used to identify tumor initiators and tumor promoters in cigarette-smoke condensates,[32] automotive exhaust emissions,[38] and urban-air particles.[14] More recently, this approach has been coupled with short-term genetic bioassays, including both microbial and mammalian-cell mutation assays, to identify mutagens and potential carcinogens in complex mixtures.[39] We first employed this method to identify the chemical classes and specific components associated with diesel particulate emissions that were mutagenic in the Ames Salmonella typhimurium mutagenesis assay.[40]

Diesel particles collected by the dilution-tunnel method[41] were Soxhlet®-extracted with dichloromethane and solvent-partitioned into organic acids, bases, and neutral components. The neutral components were further fractionated into paraffins (hexane), aromatics (1% ether/hexane to elute blue fluorescent material), transitional compounds (yellow non-fluorescent band eluted with 1% ether/hexane), and oxygenated compounds (50% acetone/methanol). The mutagenic activity of each fraction was determined using the Ames Salmonella typhimurium/-microsome assay in TA1535, TA1537, TA1538, TA98, and TA100[40]. The distributions of the mass of each fraction and of its mutagenic activity in TA98 are shown in Table 1 for the four-stroke V-8 Caterpillar 3208 engine used in urban service vehicles. The moderately and highly polar neutral compounds in the transitional (TRN) and oxygenated (OXY) fractions account for 89-94% of the mutagenic activity of the extractable organics and only 32% of the mass. Conventional gas chromatography/mass spectroscopy identified many nonmutagenic fluorenones and methylated fluorenones as major constituents of these fractions. None of these or other identified constituents accounted for the direct-acting frameshift mutagenic activity observed. Studies with nitroreductase-deficient strains of Salmonella typhimurium showed a reduction in the mutagenicity of these organics, suggesting that nitrated compounds are present.[42] Nitrated polycyclic aromatic hydrocarbons (NO_2-PAH's) are potent direct-acting frameshift mutagens detected in xerographic toners.[43] A series of NO_2-PAH's in diesel extracts were then identified and quantitated in order to estimate their contribution to the mutagenic activity of diesel particulate emissions.[44] Similar studies have also been conducted by Schuetzle.[45] These studies show that NO_2-PAH's, di-NO_2-PAH's, and hydroxyNO_2-PAH's together account for much of the mutagenicity observed in Salmonella typhimurium. Particulate emissions from catalyst-equipped gasoline-engine vehicles using unleaded fuel contain signifi-

cantly less of these NO_2-PAH's.[44] The mutagenic activity of both leaded- and unleaded-gasoline emissions is substantially increased with the addition of an exogenous metabolic activation (MA) system, suggesting that the classical PAH's may play a more important role than do NO_2-PAH's in the mutagenicity and carcinogenicity of gasoline emissions.[46]

Characterization of the mutagenicity of emissions from both vegetative and fossil fuels show general similarities. Comparison of the distribution of mutagenic activity in different organic fractions extracted from an airtight woodstove burning pine and oak are compared to the emissions from a light-duty diesel vehicle in Table 2. In both wood and diesel combustion, 82-99% of the mutagenicity was in the neutral fraction. Very little mass or mutagenic activity was observed in the organic bases. Differences in these sources were observed in the distribution of mass and mutagenicity in the neutral subfractions.

Bioassay-directed fractionation and chemical characterization has also been used to characterize and compare the complex organic emissions from roofing tar pots, coke ovens, and cigarette smoke. To obtain a gross characterization of the chemical classes present in the samples, the chemical class distribution was determined by solvent partitioning the organics into acidic, basic, neutral, and cyclohexane insoluble (a highly polar fraction) fractions. The neutral fractions were further separated into the nonpolar neutrals, aromatics (nitromethane soluble) and polar neutrals. Characterization of the distribution of mutagenic activity (Ames Salmonella typhimurium bioassay) in each of these fractions[47] showed significant differences between the diesel, coke oven main, roofing tar, and cigarette smoke condensate samples. In the diesel samples, over 90% of the mutagenic activity is located in the aromatic and polar-neutral fractions, and a significant portion of this activity can be accounted for by NO_2-PAH's. The cigarette smoke condensate, coke oven main, and roofing tar

samples did not contain detectable amounts of NO_2-PAH's.[48] Most of the mutagenicity of coke oven main sample was found in the basic fraction (37%) and polar neutral fraction (39%). The cigarette smoke condensate sample also had significant activity in the basic fraction (66%), but chemical analysis indicated that the components differed significantly from those of the coke oven main sample.[49] The roofing tar sample contained aromatic (14%) and polar (75%) mutagenic constituents that were not NO_2-PAH's. The PAH subfraction of each of these samples accounted for only a small portion of the mutagenicity (eg. diesel (0.2%), cigarette smoke condensate (0.1%), roofing tar (5%) and coke oven main (8%)).

Although the specific mutagens in these different sources are not identical, they all cause frameshift mutations and appear to be compounds that could be classified as polycyclic organic matter (POM). Chemical characterization suggests that in addition to nitrated NO_2-PAH's found in the slightly and moderately polar neutrals, hydroxylated and carboxylated polycyclic organics are found in the organic acid fraction, aromatic amines and nitrogen heterocycles are found in the organic bases and highly oxygenated quinones, diones, and nitro-oxygenated compounds are found in the polar neutral fractions.

RELATIVE MUTAGENIC AND CARCINOGENIC ACTIVITY OF COMBUSTION EMISSIONS

Mutagenic Activity

The mutagenic activity of dichloromethane extractable organics from diluted and filtered particulate emissions from different combustion sources has been evaluated in several short-term genetic bioassays. The short-term *in vitro* bioassays were performed according to standardized protocols, and when possible,

in such a way that dose-response data demonstrated a positive increase (slope) above spontaneous levels for at least three doses. When such dose-response data were obtained, the slope of the response curve was determined using simple linear regression analysis. In several short-term bioassays described elsewhere[50,51] difficulties were encountered in obtaining good dose-response data which may be due in part to solubility problems in the aqueous cell culture media. In the following bioassays, dose-dependent increases in response were obtained for many of the combustion emission samples evaluated:

1. Reverse mutation in Salmonella typhimurium TA98 (Ames bioassay)[52]
2. Forward mutation at the thymidine kinase locus in L5178Y mouse lymphoma cells (mouse lyphoma bioassay)[53]
3. Sister chromatid exchange (SCE) in Chinese hamster ovary (CHO) cells (SCE bioassay)[53]
4. Enhancement of viral (simian adenovirus SA7) transformation in Syrian hamster embryo (SHE) cells (viral enhancement bioassay)[50]

Of the above four in vitro bioassays, the first three provided reproducible dose responses. The enhancement of viral transformation assay produced variable dose responses that may have been due to variation in primary embryo cell isolations.[54]

For the Ames, mouse lymphoma, and SCE bioassays, one or more preliminary range-finding assays were performed to determine the optimum dose range for defining the slope of the dose response. The bioassays were generally performed with duplicate or triplicate treatments at five to seven doses.

Although alternative methods of analyzing the dose-response data from short-term bioassays have been reported,[55] the linear slope of the dose-response determined from simple regression analysis is presented here for the Ames, mouse lymphoma, and SCE bioassays. The dose-response slopes were uniformly consistent with a linear nonthreshold pattern. In the Salmonella typhimurium

bioassay, toxic doses (generally the highest two doses) were deleted from the regression analysis. In the three mammalian cell bioassays, doses that resulted in survivals below 10% were deleted. When data from several experiments were available, the data resulting in the best dose response as measured by the correlation coefficient (>0.8) were utilized in this study.[19] A determination of the coefficient of variation between separate bioassay slopes for the same sample has been reported elsewhere[19,56] and generally averaged 10-20%.

The slopes of each of these dose-response curves for each complex emission sample are summarized in Table 4. The diesel samples did not require an exogenous metabolic activation (MA) system in either the mouse lymphoma or the Ames bioassay, and in most cases, the addition of MA decreased the slope slightly. This was also true for the utility coal combustion emissions. The three human carcinogens, on the other hand, either required MA or were significantly enhanced in genetic activity as a result of the addition of MA. The emissions from gasoline powered vehicles, airtight woodstoves, and residential oil heaters were also enhanced by the addition of MA.

Carcinogenic Activity

Mouse skin tumorigenesis assays have had considerable use in determining the tumorigenic and carcinogenic effects of chemicals and complex mixtures.[57,58] Skin tumor initiation experiments were performed with the extractable organics from combustion particulate emissions in SENCAR mice. After administration of five single initiating doses (80 mice/dose), the mice were treated twice weekly with 12-O-tetradecanoylphorbol-13-acetate (TPA, 2.0 µg). Mice were scored individually at six months for papillomas and at one year for squamous cell carcinomas.[33,34] The carcinoma responses in the initiation assay were correlated with the papilloma responses; only the papilloma responses are referred to here.

A linear dose-response relationship for skin tumor initiation was observed for all samples up to a dose level of 1 mg. The extractable organics from fossil fuel combustion showed very similar potencies (0.12-0.59 papillomas/mouse/mg), however, the cigarette smoke condensate was much weaker (0.0024 papillomas/mouse/mg), and the coke oven samples were more potent (2.1 and 3.2 papillomas/mouse/mg) than the combustion emissions examined.

Human lung cancer unit risk estimates shown in Table 4 have been used as a basis of comparing carcinogenic potency for complex organic emissions containing polycyclic organic matter. The unit cancer risk is predicated on the linear non-threshold extrapolation model and is the individual lifetime excess lung cancer risk from continuous exposure to 1 µg carcinogen per m^3 inhaled air. The human lung cancer unit risks obtained from the epidemiologic data for coke oven workers, roofing tar applicators, and cigarette smokers were, respectively, 9.3×10^{-4}, 3.6×10^{-4}, and 2.2×10^{-6} per µg particulate organics per m^3 air.

Comparison of the Relative Potencies

The relative potencies of each of these extractable organics from combustion emissions can be directly compared to each other and to the human carcinogens by normalizing the slope data from each bioassay to one of the samples. The coke oven topside sample was selected and given a relative potency of 1.0. The relative potencies for the other samples were determined by normalizing to the coke oven topside activity for that assay (relative potency = slope normalized to coke oven topside = slope sample ÷ by slope of coke oven topside for each assay). The relative potencies are shown in Table 5.

The relative potency of the combustion emissions ranged from only a five-fold difference in the mouse skin tumorigenesis assay to a 150-fold difference in the mouse lymphoma gene mutation assay. The Ames Salmonella typhimurium mutagenesis assay showed approximately two orders of magnitude difference (100-

110-fold) from the weakest (wood and diesel combustion emissions) to the most potent (diesel and leaded gasoline samples). The cigarette smoke condensate, however was nearly three orders of magnitude less tumorigenic than the coke oven topside sample in the mouse skin tumorigenesis assay but not in the short-term bioassays. In the short-term bioassays the potency of the cigarette smoke condensate compared to coke oven topside was several orders of magnitude higher than that indicated by the epidemiologic data (Table 5). The relatively higher potency of the cigarette smoke condensate in the in vitro bioassays may be due to the presence of highly mutagenic heterocyclic amines (2-amino-9H-pyrido[2,3-b]indole and 2-amino-3methyl-9H-pyrido[2,3-b]indole).[59]

The mouse skin tumor initiation bioassay and mouse lymphoma bioassay (+MA) agreed closely on the relative potency of the Nissan diesel extract with respect to the coke oven topside sample. The Ames Salmonella typhimurium TA98 bioassay and the mouse lymphoma bioassay (-MA) yielded a higher relative potency by approximately one order of magnitude when compared to the skin tumor initiation bioassay (Table 5). The relatively high mutagenic activity of the diesel particulate extracts in the Ames bioassay compared to the human carcinogens may result from the presence of NO_2-PAH's in the diesel emissions. NO_2-PAH's have been estimated to account for a substantial portion of the Ames activity observed in diesel particulate extracts.[44] The decreased mutagenic activity of these diesel emissions in nitroreductase-deficient strains of Salmonella typhimurium[42] is additional evidence for the involvement of bacterial nitroreduction in the bacterial mutagenicity of diesel emissions.

Table 5 also presents the best estimates for the unit risk of lung cancer from epidemiologic data for roofing tar and cigarette smoke normalized to the coke oven topside sample. Roofing tar has about one-third the potency of coke oven topside, and cigarette smoke condensate had about one-four-hundredth the

potency of coke oven topside. Mouse skin initiation data compared well with the human epidemiologic data, with roofing tar and cigarette smoke at about one-fifth and one-thousandth the potency of coke oven topside, respectively.

Comparison of Mutagenic Emission Rates for Various Combustion Sources

Since the mutagenic activity (potency) of the extractable organics from combustion particulate emissions from various sources were generally within two orders of magnitude (10^2) in order to directly compare the sources, organic and particulate emission rates must be considered. The emission rates for the particle-bound organics from these sources differ in some cases by as much as 5×10^5. To compare the mutagenicity of emissions from various sources, it is critical to compare the rates of mutagenic emissions on the same consumption, or yield as shown in Table 6. To compare rates of mutagenic emissions of mobile sources, the mutagenic activity per kilometer is often reported. The rates of mutagenic emissions from stationary sources are compared on the basis of fuel consumption or energy consumption. Although stationary and mobile sources could be directly compared on a fuel-consumption basis, the distribution of fuel is fixed by consumption demands based on the relative needs for transportation, home heating, or energy.

ESTIMATION OF CANCER RISK FROM COMBUSTION EMISSIONS BASED ON COMPARATIVE POTENCY

Due to the chemical nature of the polycyclic organics produced during combustion of many types of fuels, it is likely that these POM's on soot particles emitted from combustion sources will all exhibit mutagenic and carcinogenic activity. This poses a difficult problem for both the scientific assessment of risk and the regulatory decision making process aimed at risk management. To

evaluate new or alternative combustion fuels and engines, a quantitative estimate of the cancer risk from these emissions is needed together with comparative estimates of cancer risks from existing technologies. We have described a comparative potency method for such a comparative cancer risk assessment[22] which is based on the hypothesis that there is a constant relative potency between two different carcinogens across different bioassay systems including animals and humans. The data base we have developed for conducting a comparative assessment of automotive (diesel and gasoline) emissions is summarized and expanded on here (Table 4) by the addition of oil, wood, and coal combustion emission data.

The underlying assumption of this comparative potency method is that the relative potency of two carcinogens in an *in vivo* or *in vitro* bioassay is directly proportional to the relative potency in humans. This assumption has been examined for three complex organic emission products from coke ovens, roofing tar, and cigarettes which are known to be human lung carcinogens.[22] For these three samples, the mouse skin tumor initiation relative potencies are within a factor of two, directly proportional to the relative potency estimated for these emissions from human lung cancer epidemiological data. The relative potencies, from the *in vitro* bioassays as compared to the human data, were similar for coke oven and roofing tar, but for the cigarette smoke condensate the *in vitro* tests predicted a higher relative potency. The mouse skin initiation bioassay was, therefore, used to determine the unit lung cancer risk for the most potent of the diesel emissions (Nissan).

In this comparative potency method, the risk of a suspect human carcinogen (e.g., diesel emissions) for which there are no epidemiologic cancer data was estimated by comparison to a known human carcinogen (e.g., coke oven emissions) according to the following equation:

$$\text{human risk}_{\text{diesel}} = \text{human risk}_{\text{coke oven}} \text{ relative bioassay potency } \frac{\text{diesel}}{\text{coke oven}} \quad (1)$$

The relative bioassay potency as expressed by the term, (bioassay potency$_{\text{diesel}}$ ÷ bioassay potency$_{\text{coke oven}}$), is obtained by the ratio of the slopes of the dose responses from the same in vitro or in vivo bioassays.

In the initial application of this method to the estimation of cancer risk from diesel emissions,[22] the unit risk of the most potent diesel sample (Nissan) was estimated using each of the human carcinogens. The estimated risks for this diesel sample ranged from 2.6×10^{-4} to 5.4×10^{-4} lifetime risk/µg organic/m^3 with an average of 4.4×10^{-4}. Since the skin tumor initiation activity of all the extractable organics from diesel and gasoline particulate emissions showed an excellent correlation with the short-term bioassay data, the human unit risk estimates for other diesel and gasoline combustion emission samples (x) were determined in relation to the average 4.4×10^{-4} lifeti isk/µg organic/m^3 estimated for the Nissan diesel sample according to the following equation:

$$\text{human risk}_x = 4.4\text{x}10^{-4} \text{ (average relative short-term bioassay potency)} \quad (2)$$

The relative bioassay potencies were determined for each of the short-term bioassays by dividing the mutagenicity (linear slope of the dose-response curve) of each combustion emission sample (x) by the mutagenicity of the diesel Nissan. The relative potencies for the mouse lymphoma, SCE, and Ames bioassays (+MA) were then averaged to obtain the average relative short-term bioassay potency term for equation (2) above. The final risk estimates based on short-term bioassays are shown in Table 7.

This method assumes that when comparing across combustion emissions with different chemical characteristics as described above, the mouse skin best

predicts the relative potency compared to humans. Within both diesel and gasoline automotive emissions, since the mouse skin tumor initiating activity correlated so well with the short-term bioassay data, it is further assumed that an average relative potency in these short-term bioassays can be used to estimate the comparative human risk. This approach is extended here to estimate the unit-risk for several gasoline non-catalyst vehicles and residential oil heater emissions. Since we also have mouse skin tumor initiating activity for many of these emissions, the unit risk estimates are also presented for these samples basing the comparison on the relative risk in mouse skin tumorigenicity according to equation (1). The relative potencies were compared to each of the human carcinogens (coke oven, roofing tar, and cigarette smoke) and the final estimates based on each human carcinogen were averaged to give the value shown in Table 7 based on skin tumor initiation.

The residential wood and utility coal combustion emissions have only been evaluated in the Ames Salmonella typhimurium mutagenesis bioassay (Table 4), therefore, a range of estimated risks is presented in Table 7 determined from the relative potency in the Ames assay compared to each of the human carcinogens. These estimates range over 2-3 orders of magnitude because of the 2-3 orders of magnitude difference in human risk between the cigarette smoke and the other human carcinogens (a difference which is not reflected in the Ames assay). Using the Ames relative potency compared to the diesel Nissan results in 0.3×10^{-4} lifetime risk/μg organic/m^3 for wood and $1\text{-}4 \times 10^{-4}$ lifetime risk/μg organic/m^3 for the coal emissions. This limited bioassay data (Ames test alone) is insufficient to select the relative bioassay data which best fits the relative potency model.

The major assumption and main source of uncertainty in this comparative potency method is the validity of the constant relative potency hypothesis.

Interspecies differences that always plague efforts to extrapolate from animals to man (e.g., differences in absorption, distribution, metabolism, DNA damage and repair capabilities) may also effect the constant relative potency. By designing studies to test this hypothesis we may be able to determine the range of variation in potency for different bioassays and emission sources. For example, it is apparent from the existing data base that the relative potency of the diesel samples in the bacterial (Ames) assay are approximately one order of magnitude greater than the relative potency in mouse skin. This difference may be due to differences between mouse skin and bacteria in their ability to metabolize chemicals present in diesel emissions (e.g., nitrated PNA). Since we have assumed that the relative potency in mouse skin tumor initiating activity best predicts the relative potency in humans, it is possible that we have underestimated the human risk by approximately 10-fold. This would be the case if future additional data suggests that bacteria more accurately reflects the relative carcinogenic potency in humans than does the mouse skin tumor initiation assay. As additional human risk and animal cancer data (e.g., from intratrachael instillation) become available, this assumption and methodology should be re-evaluated.

When extrapolating from animal cancer data to human cancer risks for a single agent using classical methodology, bioavailability, tissue distribution, metabolism and target organ dosimetry data are utilized to relate exposure to dose. The comparative potency method does not eliminate the utility of such data but does provide a method for estimating risk with the simplified assumption that these factors are directly proportional or constant from one bioassay to another. Under this assumption, for example, the relative bioavailability of carcinogens from diesel particles in humans compared to coke oven particles would be directly proportional to the bioavailability of these carcinogens in

the non-human bioassays. The most useful data to test this assumption would be the relative bioavailability of carcinogens from these different particles in different bioassays and in humans. Since such data are not now available, the validity of this assumption remains untested.

The constant relative potency model does not assume that the carcinogens being compared are chemically related. Future evaluation of this model may show that it is only valid for either very similar chemicals or for combustion emissions where the carcinogens present in these mixtures are identical and vary only in their concentration. Chemical characterization of the different complex mixtures show that all of the mixtures contain POMs which cause frame-shift mutations in Salmonella typhimurium and in that sense these emissions are similar. Upon chemical fractionation, however, significant differences were observed in the distribution of mutagenic activity in various fractions indicating that although these compounds may generally be polycyclic aromatic compounds, the specific mutagenic chemicals are different (e.g., by substitution of nitro, amino, or oxygenated moieties).

SUMMARY

Short-term genetic bioassays have been useful in both characterizing and evaluating the complex organic emissions from many combustion sources. Identification of the specific mutagens and carcinogens in these complex organics has been greatly facilitated by the use of bioassay-directed chemical fractionation and characterization methods. Although all combustion emissions appear to contain neutral polycyclic organic compounds which are mutagenic and tumorigenic, the classical PAH's do not account for all of the potential carcinogens in these emissions. Substituted and oxidized PAH's appear to play a significant

role in the mutagenic and tumorigenic activity of many of these combustion emissions.

Studies on the relative mutagenic and carcinogenic activity of the extractable organics from diluted and filtered soot particles, show that these organics from very different combustion sources differ at the most by two orders of magnitude (10^2). Comparison of mutagenic and organic emission rates for these different sources differ by four to five orders of magnitude (10^4-10^5). Utilizing a recently developed approach to evaluating the comparative potency of these emissions, the estimated cancer risk from the various combustion particles is similar and ranges only over two orders of magnitude except for utility power plants which have very low organic emission rates due to the high combustion efficiency of these sources.

DISCLAIMER

The research described in this paper has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

REFERENCES

1. Kennaway, E. L., Experiments on cancer producing substances, Brit. Med. J., 1, 1, 1925.
2. Cook, J. W., Hieger, J., Kennaway, E. L., and Mayneord, W. V., The production of cancer by pure hydrocarbons, Proc. Roy. Soc. Biol., 111, 455, 1932.

3. Cook, J. W. and Kennaway, E. L., Chemical compounds as carcinogenic agents, Amer. J. Cancer, 33, 50, 1938.

4. Dipple, A., Polynuclear aromatic carcinogens, in Chemical Carcinogens, ACS Monograph 173, Searle, C. E., Ed., American Chemical Society, Washington, D.C., 1976, 245.

5. Shabad, L. M., Carcinogenic action of polycyclic hydrocarbons in animals and man, in Air Pollution and Cancer in Man, Vol. 16, Mohr, U., Schmahl, D. and Tomatis, L., Eds., IARC Scientific Publication, Lyon, 1977, 257.

6. IARC, IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man, Certain polycyclic aromatic hydrocarbons and heterocyclic compounds, Vol. 3, International Agency for Research on Cancer, Lyon, 1973.

7. Walter, R., The benzpyrene of town air, Brit. J. Cancer, 6, 8, 1952.

8. Pike, M. C., Gordon, R. J., Henderson, B. E., Menck, H. R., and Soo Hoo, J., Air pollution in Persons at High Risk of Cancer, Fraumeni, J. F., Ed., Academic Press, New York, 1975, 225.

9. National Research Council: Particulate Polycyclic Organic Matter. National Academy of Sciences, Washington, D.C., 1972.

10. Bjorseth, A., Determination of polynuclear aromatic hydrocarbons in the working environment, in Polynuclear Aromatic Hydrocarbons, Jones, P. W. and Leber, P., Eds., Ann Arbor Science, Ann Arbor, 1979, 371.

11. Bjorseth, A., Analysis of polycyclic aromatic hydrocarbons in particulate matter by glass capillary gas chromatography, Anal. Chem. Acta., 94, 21, 1977.

12. Speizer, F. E., Assessment of the epidemiological data relating lung cancer to air pollution, Environ. Health Persp., 47, 33, 1983.

13. Claxton, L. D., Review of fractionation and bioassay characterization techniques for the evaluation of organics associated with ambient air particles,

in Genotoxic Effects of Airborne Agents, Tice, R. R., Costa, D. L., Schaich, K. M., Eds., Plenum Press, New York, 1982, 19.

14. Hueper, W. C., Kotn, P., Tabor, E.C., Payne, W. W., Falk, H., and Sawicki, E., Arch. Pathol., 74, 89, 1962.

15. Alfheim, I., Lofroth, G. and Moller, M., Bioassay of extracts of ambient particulate matter, Environ. Health Persp., 47, 227, 1983.

16. Lewtas, J., Austin, A., Claxton, L., Burton, R. and Jungers, R., The relative contribution of PNA's to the microbial mutagenicity of respirable particles from urban air, in Polynuclear Aromatic Hydrocarbons, Jones, P. W. and Leber, P., Eds., Ann Arbor Science, Ann Arbor, 1979, 449.

17. Lewtas, J., Comparison of the mutagenic and potentially carcinogenic activity of particle bound organics from wood stoves, residential oil furnaces, and other combustion sources, in Residential Solid Fuels, Cooper, J. A., Malik, D., Eds., Oregon Graduate Center, Oregon, 1982, 606.

18. Mumford, J. and Lewtas, J., Mutagenicity and cytotoxicity of coal fly ash from fluidized-bed and conventional combustion, J. Tox. and Environ. Health, 10, 565, 1982.

19. Lewtas, J., Evaluation of the mutagenicity and carcinogenicity of motor vehicle emissions in short-term bioassays, Environ. Health Persp., 47, 141, 1983.

20. Lewtas, J., Bradow, R. L., Jungers, R. H., Harris, B. D., Zweidinger, R. B., Cushing, K. M., Gill, B. E., and Albert, R. E., Mutagenic and carcinogenic potency of extracts of diesel and related environmental emissions: study design, sample generation, collection and preparation, Environ. Int., 5, 383, 1981.

21. Nesnow, S. and Lewtas, J., Mutagenic and carcinogenic potency of extracts of diesel and related environmental emissions: summary and discussion of

the results, Environ. Int., 5, 425, 1981.

22. Albert, R. E., Lewtas, J., Nesnow, S., Thorslund, T. W., and Anderson, E., A comparative potency method for cancer risk assessment: Application to diesel particulate emissions, Risk Analysis, in press.

23. Pitts, J. N., Formation and fate of gaseous and particulate mutagens and carcinogens in real and simulated atmospheres, Environ. Health Persp., 47, 115, 1983.

24. Levin, W., Wood, A. W., Yogi, H., Dansette, P. M., Jerina, D. M., and Couney, A. H., Carcinogenicity of benzo(a)pyrene-4,5-, 7,8-, and 9,10-oxides on mouse skin, Proc. Natl. Acad. Sci. (U.S.), 73, 243, 1976.

25. Sugimura, T. and Takayama, S., Biological actions of nitroarenes in short-term tests on Salmonella, cultured mammalian cells and cultured human trachael tissues: possible basis for regulatory control, Environ. Health Persp., 47, 171, 1983.

26. Holmberg, B. and Ahlborg, U., Consensus report: Mutagenicity and carcinogenicity of car exhausts and coal combustion emissions, Environ. Health Persp., 47, 1, 1983.

27. Rudling, L., Ahling, B., and Lofroth, G., Chemical and biological characterization of emissions from combustion of wood and wood-chips in small furnaces and stoves, in Residential Solid Fuels, Cooper, J. A. and Malek, D., Eds., Oregon Graduate Center, Oregon, 1982, 34.

28. Wolff, G. T. and Klimisch, R. L., Particulate Carbon: Atmospheric Life Cycle, Plenum Press, New York, 1982.

29. Alfheim, I., Bergstrom, J. G. T., Jensson, D. and Moeller, M., Mutagenicity in emissions from coal- and oil-fired boilers, Environ. Health Persp., 47, 177, 1983.

30. Rannug, U., Data from short-term tests on motor vehicle exhausts, Environ.

Health Persp., 47, 177, 1983.

31. Merrill, R. G., Jr., Lewtas, J., and Hall, R. E., Source assessment sampling system (SASS) versus dilution tunnel sampling, in Short-Term Bioassays in the Analysis of Complex Environmental Mixtures III, Waters, M. S., Sandhu, S. S., Lewtas, J., Claxton, L., Chernoff, N. and Nesnow, S., Eds., Plenum Press, New York, 1983, 17.

32. Hoffmann, D. and Wynder, E. L., Environmental respiratory carcinogenesis, in Chemical Carcinogens, ACS Monograph 173, Searle, C. E., Ed., American Chemical Society, Washington, D.C. 1976, chap. 7.

33. Nesnow, S., Triplett, L., and Slaga, T. J., Comparative tumor-initiating activity of complex mixtures from environmental particulate emissions on SENCAR mouse skin, J. Natl. Cancer Inst., 68, 829, 1982.

34. Nesnow, S., Evans, C., Stead, A., Creason, J., Slaga, T. J., and Triplett, L. L., Skin carcinogenesis studies of emission extracts, in Toxicological Effects of Emissions from Diesel Engines, Lewtas, J., Ed., Elsevier Science Publishing Co., Inc., New York, 1982, 295.

35. Thilly, W. G., Longwell, J. and Andon, B. M., A general approach to the biological analysis of complex mixtures in combustion effluents, Environ. Health Persp., 48, 129, 1983.

36. Schuetzle, D., Lee, F. S. C., Prater, T. J. and Tejada, S. B., The identification of polynuclear aromatic (PAH) derivatives in mutagenic fractions of diesel particulate extracts, Int. J. Environ. Anal. Chem., 9, 93, 1981.

37. Rappaport, S. M., Wang, Y. and Wei, E. T., Sawyer, R., Watkings, B. E. and Rappaport, H., Identification of a direct-acting mutagen in diesel exhaust particulates, Environ. Sci. Technol., 14, 1505, 1980.

38. Grimmer, G., Naujack, K.-W., Dettbarn, G., Brune, H., Deutsch-Wenzel, R., and Misfeld, J., Analysis of balance of carcinogenic impact from emission

condensates of automobile exhaust, coal heating, and used engine oil by mouse-skin-painting as a carcinogen-specific detector, in Polynuclear Aromatic Hydrocarbons, Cooke, M., Dennis, A. J., and Fisher, G. L., Eds., Battelle-Columbus Press, Columbus, 1982, 335.

39. Epler, J. L., Clark, B. R., Ho, C.-h., Guerin, M. R., and Rao, T. K., Short-term bioassay of complex organic mixtures: part II, mutagenicity testing, in Application of Short-Term Bioassays in the Fractionation and Analysis of Complex Environmental Mixtures, Waters, M. D., Nesnow, S., Huisingh, J. L., Sandhu, S. S., and Claxton, L., Eds., Plenum Press, New York, 1978, 269.

40. Huisingh, J., Bradow, R., Jungers, R., Claxton, L., Zweidinger, R., Tejada, S., Bumgarner, J., Duffield, F., Waters, M., Simmon, V. F., Hare, C., Rodriguez, C., and Snow, L., Application of bioassay to the characterization of diesel particle emissions, in Application of Short-Term Bioassays in the Fractionation and Analysis of Complex Environmental Mixtures, Waters, M. D., Nesnow, S., Huisingh, J. L., Sandhu, S. S., and Claxton, L., Eds., Plenum Press, New York, 1978, 381.

41. Bradow, R. L., Diesel particle and organic emissions: engine simulation, sampling, and artifacts, in Toxicological Effects of Emissions from Diesel Engines, Lewtas, J., Ed., Elsevier Science Publishing Co., Inc., New York, 1982, 33.

42. Claxton, L. D., and Huisingh, J. L., Comparative mutagenic activity of organics from combustion sources, in Pulmonary Toxicology of Respirable Particles, Sanders, C. L., Cross, F. T., Dagle, G. E., Mahaffey, J. A., Eds., Department of Energy, U.S. Government Printing Office, CONF-791002, 1980, 453.

43. Rosenkranz, H. S., McCoy, E. C., Sanders, D. R., Butler, M., Kiriazides,

D. K., and Mermelstein, R., Nitropyrenes: isolation, identification, and reduction of mutagenic impurities in carbon black and toners, Science, 209, 1039, 1980.

44. Nishioka, M. G., Petersen, B. A., and Lewtas, J., Comparison of the nitroaromatic content and mutagenicity of diesel emissions, in Polynuclear Aromatic Hydrocarbons, Cooke, M., Dennis, A. J. and Fisher, G. L., Eds., Battelle-Columbus Press, Columbus, 1982, 603.

45. Schuetzle, D., Sampling of vehicle emissions for chemical analysis and biological testing, Environ. Health Persp., 47, 65, 1983.

46. Lewtas, J., Mutagenic activity of diesel emissions, in Toxicological Effects of Emissions from Diesel Engines, Lewtas, J., Ed., Elsevier Science Publishing Co., Inc., New York, 1982, 243.

47. Austin, A., Claxton, L. and Lewtas, J., Mutagenicity of particle-bound organic chemical fractions from diesel and comparative emissions, in Diesel Emissions Symposium Proceedings, EPA-600/9-82-014, National Technical Information Service, Springfield, 1982, 478.

48. Nishioka, M. and Petersen, B. A., personal communications.

49. Sparacino, C., Williams, R., Brady, K., and Jungers, R., Fractionation and characterization of the organics from diesel and comparative emissions, in Diesel Emissions Symposium Proceedings, EPA-600/9-82-014, National Technical Information Service, Springfield, 1982, 598.

50. Casto, B. C., Hatch, G. G., Huang, S. L., Huisingh, J. L., Nesnow, S., and Waters, M. D., Mutagenic and carcinogenic potency of extracts of diesel and related environmental emissions: in vitro mutagenesis and oncogenic transformation, Environ. Int., 5, 403, 1981.

51. Curren, R. D., Kouri, R. E., Kimi, C. M., and Schechtman, L. M., Mutagenic and carcinogenic potency of extracts from diesel related environmental

emissions: simultaneous morphological transformation and mutagenesis in BALB/c 3T3 cells, Environ. Int., 5, 411, 1981.

52. Claxton, L. D., Mutagenic and carcinogenic potency of diesel and related environmental emissions: Salmonella bioassay, Environ. Int., 5, 389, 1981.
53. Mitchell, A. D., Evans, E. L., Jotz, M. M., Riccio, E. S., Mortelmans, K. E., and Simmon, V. F., Mutagenic and carcinogenic potency of extracts of diesel and related environmental emissions: in vitro mutagenesis and DNA damage, Environ. Int., 5, 393, 1980.
54. Pienta, R. J., A hamster embryo cell model system for identifying carcinogens, in Carcinogenesis: Identification and Mechanisms of Action, Griffin, A. C. and Shaw, C. R., Eds., Raven Press, New York, 1979, 121.
55. Stead, A. G., Hasselblad, V., Creason, J. P., and Claxton, L., Modeling the Ames test, Mutat. Res., 85, 13, 1981.
56. Claxton, L. and Kohan, M., Bacterial mutagenesis and the evaluation of mobile-source emissions, in Short-Term Bioassays in the Analysis of Complex Environmental Mixtures II, Waters, M. D., Sandhu, S. S., Huisingh, J. L., Claxton, L., and Nesnow, S., Eds., Plenum Press, New York, 1981, 299.
57. Nesnow, S., Triplett, L. L. and Slaga, T. J., Mouse skin tumor initiation-promotion and complete carcinogenesis bioassays: mechanisms and biological activities of emission samples, Environ. Health Persp., 47, 255, 1983.
58. Pereira, M. A., Skin tumorigenesis research data base, J. Amer. Coll. Toxicol., 1, 47, 1982.
59. Sugimura, T., A view of a cancer researcher on environmental mutagens, in Proceedings of the Third International Conference on Environmental Mutagens, Tokyo, Japan, in press.
60. Harris, J. E., Diesel emissions and lung cancer, Risk Analysis, in press.
61. Dudney, C. S., Walsh, P. J., Jones, T. D., Calle, E. E., and Griffin, G. D.,

On the use of relative toxicity for risk estimation, in Short-Term Bioassays in the Analysis of Complex Environmental Mixtures III, Waters, M. D., Sandhu, S. S., Lewtas, J., Claxton, L., Chernoff, N. and Nesnow, S., Eds., Plenum Press, New York, 1983, 499.

62. Petersen, B. A. and Chuang, C. C., Methodology of fractionation and partition of diesel exhaust particulate samples, in Toxicological Effects of Emissions from Diesel Engines, Lewtas, J., Ed., Elsevier Science Publishing Co., Inc., New York, 1982, 51.

TABLE 1

Distribution of the Mass and Mutagenic Activity of Fractionated Diesel Particle Organics[a]

Fraction[b]	Mass (%)	Specific Mutagenic Activity[c] (rev/mg)		Weighted Mutagenic Activity[d] (rev/mg)		Distribution of Mutagenic Activity[e] (%)	
		-MA	+MA	-MA	+MA	-MA	+MA
Organic acids	14.9	193	248	28.8	37.0	4.9	9.5
Organic bases	0.3	43.8	132	0.13	0.40	0.02	0.10
Ether insolubles	3.9	53.9	80.9	2.1	3.2	0.36	0.80
Paraffins	36.7	Neg.	Neg.	0.0	0.0	0.0	0.0
Aromatics	6.9	49.5	30.1	3.42	2.1	0.60	0.54
Transitionals	5.0	7520	2620	376	131	64.9	33.5
Oxygenates	26.9	629	798	169	215	29.2	55.4
Sum				579	389		

[a]Diesel particles obtained by the dilution tunnel method from a four-stroke V-8 Caterpillar 3208 engine operated on the standard 13 mode series of steady states.[40]

[b]Dichloromethane extractable organics were solvent-partitioned into acids, bases, and neutrals as previously described.[40] The neutral fraction was further fractionated on silica gel into four fractions by the following solvents: hexane (paraffins), 1% ether in hexane (aromatics and transitionals), and 50% acetone in methanol (oxygenates).

[c]*Salmonella typhimurium* mutagenesis assay performed with and without metabolic activation (MA). Slope determined from linear regression analysis of the initial portion of the dose-response curve.

[d]Weighted mutagenic activity of each fraction relative to the total extract is the product of distribution of mass in each fraction (% mass) and the specific mutagenic activity (rev/mg). The sum of these weighted mutagenic activities is used to determine the distribution of mutagenicity (%) as described below.

[e]Distribution of mutagenic activity is the percentage of weighted mutagenic activity in each fraction compared to the sum of the weighted mutagenic activities. This distribution assumes that any mutagenicity not recovered through the fractionation is equally distributed across all fractions.

TABLE 2

Characterization of Mutagenicity from Combustion of Pine and Oak in an Airtight Woodstove Compared to Diesel Combustion Emissions

Fraction[a]	Diesel[b]		Pine[c]		Oak[c]	
	mass %	mutagenicity[d] %	mass %	mutagenicity[b] %	mass %	mutagenicity[b] %
1. organic acids	7.6	17.9	47.0	0.0	44.0	0.0
2. organic bases	0.2	<0.1	2.3	1.3	2.2	4.8
3. neutrals	(92.2)[e]	(82.1)	50.7	98.7	53.7	95.2
A. aliphatics (hexane)	56.1	0.0	14.3	10.9	14.2	29.4
B. aromatics (hexane/benzene)	12.4	37.0	19.5	14.7	18.1	23.7
C. moderately polar (dichloromethane)	3.1	24.7	20.1	70.2	29.0	11.6
D. highly polar (methanol)	20.6	20.4	46.1	4.2	38.7	35.3

[a]The acids and bases were first separated by liquid-liquid partitioning to separate the acids and bases. The neutrals are partioned into four fractions by open column chromatography on 5% water deactivated silica gel utilizing the solvents designated in parenthesis.[62]

[b]Diesel particles collected by the dilution tunnel technique from a 1973 Datsun Nissan 220C diesel automobile operated on a chassis dynamometer using the highway fuel economy test cycle (HWFET).[20] The total exhaust was diluted (10:1) with filtered air prior to collection on 20 x 20 inch Teflon coated Pallflex T60-A20 filters. The particles were Soxhlet extracted with dichloromethane (DCM) for 48 hrs prior to fractionation.

[c]Pine and oak combustion particles collected by the dilution tunnel technique from an airtight woodstove (Johnson Energy Converter) during the constant burn phase.[17] The emissions were diluted, collected, and Soxhlet extracted by the same techniques employed for the diesel particles.

[d]Data from the Salmonella typhimurium plate incorporation assay in TA98 with metabolic activation was analyzed by the model slope method[19] to determine the specific mutagenic activity in revertants/µg of fraction tested. Distribution of mutagenic activity between each fraction (% mutagenicity) is the percentage of weighted mutagenic activity [mass (%) x specific mutagenic activity (rev/µg) of each fraction] of each fraction compared to the sum of the weighted mutagenic activities.

[e]Sum of the neutral subfractions shown in parenthesis.

TABLE 3

Comparison of the Distribution of Mutagenic Activity of Different Sources

Fraction[a]	Diesel[b]		Cigarette Smoke[c]		Coke Oven[d]		Roofing Tar[e]	
	mass %	muta-genicity[f] %	mass %	muta-genicity %	mass %	muta-genicity %	mass %	muta-genicity %
1. organic acids (A)	3.6	4.0	1.0	0.7	0.3	0.1	2.3	1.1
2. organic bases (B)	1.0	3.8	12.2	66.1	3.9	37.1	2.9	5.9
3. neutrals (sum of A, B,C) (N)	(95.4)	(92.1)	(86.8)	(33.1)	(95.8)	(62.8)	(94.8)	(93.0)
A. non-polar neutrals (NPN) (cyclohexane soluble)	75.1	1.4	0.6	2.2	13.9	6.6	40.4	3.9
B. aromatics (sum 1-4) (ARM) (nitro-methane soluble)	(4.5)[g]	(21.8)	(0.6)	(5.8)	(17.8)	(17.0)	(33.3)	(13.9)
1. PNA's (PNA1)	1.1	0.2	<0.1	0.1	9.0	7.9	31.8	4.9
2. slightly polar aromatics (PNA2)	1.4	13.6	0.0	0.0	0.0	0.0	1.2	7.9
3. moderately polar aromatics (PNA3)	0.7	7.8	<0.1	0.1	7.4	5.4	<0.1	0.1
4. highly polar aromatics (PNA4)	1.3	0.2	0.5	5.6	1.4	3.7	0.3	1.0
C. polar neutrals (sum 1&2)	(15.8)	(68.9)	(85.6)	(25.1)	(64.1)	(39.2)	(21.1)	(75.2)
1. methanol soluble (PN)	7.7	52.5	11.6	0.0	6.5	8.2	13.1	17.3
2. cyclohexane in-soluble (CI)	8.1	16.4	74.0	25.1	57.6	31.0	8.0	57.9

Footnotes for Table 3.

[a]Fractionation[49] by liquid-liquid partioning into the following fractions: bases (10% and 20% sulfuric acid), acids (1 N. sodium hydroxide), nonpolar neutrals (cyclohexane), aromatics (nitromethane), polar neutrals (methanol and water), and cyclohexane insolubles. The aromatics were further fractionated by preparative silica gel chromatography using sequentially more polar solvents to obtain the following fractions: PNA 1 (2% dichloromethane in hexane, PNA 2 (50% dichloromethane in hexane), PNA 3 (100% dichloromethane), PNA 4 (methanol in dichloromethane).

[b]Diesel particles from a 1978 Oldsmobile 350 diesel automobile, collected and extracted as described in Table 2.

[c]Cigarette smoke condensate from Kentucky Reference 2R1 cigarette collected as described previously.[20]

[d]Coke oven mains collected from a separator located between the gas collector main and primary coolers within the coke oven battery.

[e]Roofing tar emissions collected from pitch based coal tar as described previously.[20]

[f]Salmonella typhimurium TA98 plate incorporation assay with Aroclor induced rat liver S9 metabolic activation system. Mutagenicity (%) distribution determined as described in Table 1.

[g]Values shown in parenthesis are calculated sums of the subfractions shown below.

TABLE 4

Comparative Mutagenicity and Carcinogenicity of Extractable Organcis

Source[a]	Human Epidemiologic Data	Bioassay Data							
		Animal in vivo	Short-Term in vitro						
	Lung Cancer Unit Risks[b]	Mouse Skin Tumor Initiation[c]	Enhancement of Viral Transformation[d]	Mutation in L5178Y Mouse Lymphoma Cells[e]		SCE in CHO Cells[f]		Ames Salmonella typhimurium TA98[g]	
				(-MA)	(+MA)	(-MA)	(+MA)	(-MA)	(+MA)
Comparative Emissions									
Human Carcinogens									
° Coke Oven Main[47,22]	--	3.2	--	0.38	27.0	0.20	0.19	0.11	6.7
° Coke Oven Topside[20]	9.3×10^{-4}	2.1	1.2	0.71	12.0	0.41	0.03	0.7	1.1
° Roofing Tar[20]	3.6×10^{-4}	0.41	0.87	0.39	17.0	0.12	0.02	Neg[h]	0.86
° Cigarette Smoke Condensate[20]	2.2×10^{-6}	0.0024	0.35	0.39	0.79	0.12	0.08	Neg	0.57
Combustion Emissions									
Diesels[19]									
° Mercedes		0.37	--	0.03	1.5	0.09	0.16	10.0	12.0
° Nissan		0.59	0.42	4.2	2.9	0.30	0.071	11.0	13.0
° Volkswagen Rabbit		0.24	0.14	0.98	0.72	0.075	0.030	3.8	3.0
° Oldsmobile		0.31	0.030	1.2	1.3	Neg	0.017	2.2	1.5
° Caterpillar		Neg	Neg	0.25	0.063	0.011	Neg	0.38	0.31
Gasoline Catalyst[19]									
° Mustang II		0.17	0.024	0.38	1.1	0.076	--	1.6	3.5
Gasoline Non-Catalyst[19]									
°Chevrolet 366		--	--	1.2	4.9	0.72	0.22	2.9	6.2
° Ford Van		--	--	2.1	5.7	0.62	0.47	17.0	32.0
Residential Heaters[17,31]									
° Oil		0.12	0.28	1.2	2.6	0.06	0.04	1.3	2.1
° Wood		--	--	--	--	--	--	0.15	0.93
Utility Power Plants[18]									
° Coal, Conventional		--	--	IP[i]	IP	IP	IP	3.1	--
° Coal, FBC		--	--	IP	IP	IP	IP	9.4	5.2

Footnotes for Table 4.

[a]References to the collection method and original bioassay dose-response data are indicated with each emission source.

[b]Lifetime risk/µg organics/m^3 calculated from the linear nonthreshold extrapolation model (Thorslund, T.W).

[c]Papillomas/mouse/mg based on papilloma multiplicity data in Sencar mice (Nesnow, S., Triplett, L.L., and Slaga, T.J.).

[d]Transformation frequency (transformed foci/10^6 surviving cells)/µg/ml.

[e]Mutation frequency (TK mutants/10^6 surviving cells)/µg/ml.

[f]SCE/cell/µg/ml, (-MA) was a 21.5 h exposure and (+MA) was a 2 hr exposure.

[g]Revertants/µg (from simple linear regression analysis).

[h]Neg = Negative.

[i]IP = In progress.

TABLE 5

Relative Mutagenicity and Carcinogenicity of Extractable Organics

Source	Human Epidemiologic Data	Bioassay Data								
		Animal Bioassay	Short-Term Bioassay							
	Lung Cancer Unit Risks[a]	Mouse Skin Tumor Initiation[b]	Enhancement of Viral Transformation[c]	Mutation in L5178Y Mouse Lymphoma Cells[d]		SCE in CHO Cells[e]		Ames Salmonella typhimurium TA98[f]	
				(-MA)	(+MA)	(-MA)	(+MA)	(-MA)	(+MA)
Comparative Emissions									
Human Carcinogens									
° Coke Oven Main	--	1.5	--	0.53	2.3	0.49	6.3	0.16	6.1
° Coke Oven Topside	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
° Roofing Tar	0.39	0.2	0.73	0.55	1.4	0.29	0.67	0.0	0.78
° Cigarette Smoke Condensate	0.0024	0.0011	0.29	0.55	0.066	0.29	2.7	0.0	0.52
Combustion Emissions									
Diesels									
° Mercedes		0.18	--	0.04	0.12	0.22	5.3	14.0	11.0
° Nissan		0.28	0.35	5.9	0.24	0.73	2.4	16.0	12.0
° Volkswagen Rabbit		0.11	0.12	1.4	0.06	0.18	1.0	5.4	2.7
° Oldsmobile		0.15	0.03	1.7	0.11	0.0	0.57	3.1	1.4
° Caterpillar		0.0	0.0	0.35	0.005	0.027	0.0	0.54	0.28
Gasoline Catalyst									
° Mustang II		0.08	0.02	0.53	0.09	0.19	--	2.3	3.2
Gasoline Non-Catalyst									
°Chevrolet 366		--	--	1.7	0.41	1.8	7.3	4.1	5.6
° Ford Van		--	--	3.0	0.48	1.5	16.0	24.0	29.0
Residential Heaters									
° Oil		0.06	0.23	1.7	0.22	0.15	1.3	1.9	1.9
° Wood		--	--	--	--	--	--	0.21	0.85
Utility Power Plants									
° Coal, Conventional		--	--	IP[h]	IP	IP	IP	4.4	--
° Coal, FBC		--	--	IP	IP	IP	IP	13.4	4.7
Range of Combustion Emissions		5	18	150	96	67	9	110	100

Footnotes for Table 5.

[a]Lifetime risk/µg organics/m^3 calculated from the linear nonthreshold extrapolation model (Thorslund, T.W).

[b]Papillomas/mouse/mg based on papilloma multiplicity data in Sencar mice (Nesnow, S., Triplett, L.L., and Slaga, T.J.).

[c]Transformation frequency (transformed foci/10^6 surviving cells)/µg/ml.

[d]Mutation frequency (TK mutants/10^6 surviving cells)/µg/ml.

[e]SCE/cell/µg/ml, (-MA) was a 21.5 h exposure and (+MA) was a 2 hr exposure.

[f]Revertants/µg (from simple linear regression analysis).

[g]Neg = Negative.

[h]IP = In progress.

TABLE 6

Mutagenic Emission Rates for Various Combustion Sources

Source	Fuel type	Mutagenicity of Organics (rev/μg)	Organic Emission Rate (mg/km)	Organic Emission Rate (mg/kg)	Organic Emission Rate (ng/J)	Mutagenic Emission Rate (rev/km)	Mutagenic Emission Rate (rev/kg)	Mutagenic Emission Rate (rev/MJ)
Diesel engine								
Car (Mercedes)	diesel	12.0	19.2			240,000		
Truck (Mack)	diesel	2.3	143.0			320,000		
Bus (GM)	diesel	0.1	357.0			37,000		
Gasoline engine								
Non-catalyst (Ford Van)	unleaded gasoline	32.0	5.7			180,000		
Catalyst (Mustang II)	leaded gasoline	3.5	1.4	43		5,000	140,000	
Residential heaters								
Woodstove	Pine	1.3		8940	508.0		12,000,000	660,000
	Oak	0.9		3096	187.0		2,800,000	168,000
Residential oil								
furnace #1	No. 2 fuel oil	2.0		21	0.5		40,000	1,000
furnace #2	No. 2 fuel oil	5.1		70	1.5		360,000	7,600
Utility power plants (coal)	coal	3.1			0.01			31

[a] *Salmonella typhimurium* TA98 with metabolic activation

TABLE 7

Application of the Comparative Potency Method to Estimation of Lung Cancer Unit Risks for Combustion Emissions

Emission Sources	Unit-Risk Estimates (Lifetime risk/μg/m^3)			
	Organics		Particles	
	Relative Potency in Skin Tumor Initiation[a]	Relative Potency in Short-Term Bioassays[b]	Relative Potency in Skin Tumor Initiation	Relative Potency in Short-Term Bioassays
DIESELS				
Nissan	4.4×10^{-4}	---	3.5×10^{-5}	---
Volkswagen Rabbit	1.7×10^{-4}	1.3×10^{-4}	3.0×10^{-5}	2.3×10^{-5}
Oldsmobile	2.4×10^{-4}	1.2×10^{-4}	3.9×10^{-5}	2.0×10^{-5}
Caterpillar	---	0.066×10^{-4}	---	0.2×10^{-5}
GASOLINE CATALYST				
Mustang II	1.2×10^{-4}	1.4×10^{-4}	5.1×10^{-5}	6.0×10^{-5}
GASOLINE NONCATLYST				
Chevrolet 366	---	7.9×10^{-4}	---	1.6×10^{-5}
Ford Van	---	16.0×10^{-4}	---	30.0×10^{-5}
RESIDENTIAL HEATERS				
Oil	0.95×10^{-4}	2.4×10^{-4}	0.91×10^{-5}	2.3×10^{-5}
Wood	---	$(0.04\text{-}8\times10^{-4})$[c]	---	$(0.4\text{-}80\times10^{-5})$[c]
UTILITY POWER PLANTS				
Coal	---	$(0.1\text{-}40\times10^{-4})$[d]	---	$(0.2\text{-}80\times10^{-8})$[d]

[a]Based on relative mouse skin tumor initiation activity (Table 5) compared to the coke oven, roofing tar, and cigarette smoke. These three unit risks (2.6×10^{-4}, 5.2×10^{-4}, and 5.4×10^{-4} respectively have been averaged.

[b]Based on average relative activity in the mouse lymphoma, SCE, and Ames bioassays (+MA) compared to the Nissan diesel sample. This is determined by the average relative potency in the mouse lymphoma, SCE, and Ames bioassay (+MA).

[c]Based on relative activity in the Ames bioassay (+MA) compared to the relative human cancer risks for coke oven (8×10^{-4}), roofing tar (4×10^{-4}), and cigarette smoke (0.04×10^{-4}). Estimation based on Nissan diesel human risk (4.4×10^{-4}) and comparison of the relative Ames bioassay (+MA) of wood compared to Ames bioassay (+MA) of Nissan diesel results in 0.3×10^{-4} lifetime risk/μg organics/m^3.

[d]Based on relative activity in the Ames bioassay (-MA) compared to the relative human cancer risks for coke oven (40×10^{-4}), roofing tar (16×10^{-4}), and cigarette smoke (0.1×10^{-4}). Estimation based on Nissan diesel human risk (4.4x10-4) and comparison of the relative Ames bioassay (-MA) of utility coal combustion compared to the Ames bioassay (-MA) of Nissan diesel results in 1×10^{-4} to 4×10^{-4} lifetime risk/μg organics/m^3.

PART II: CONTRIBUTION OF NO_2-PAHS TO THE MUTAGENIC ACTIVITY OF AUTOMOTIVE EMISSIONS AND URBAN AIR PARTICLES

Several lines of evidence suggest that nitrated polynuclear aromatic hydrocarbons (NO_2-PAHs) are responsible for much of the "direct-acting" bacterial mutagenicity of extracts of diesel exhaust particules and urban air particulate matter.[1] Quantification of the concentration of a series of NO_2-PAHs and determination of their contribution to the direct-acting mutagenicity in Salmonella typhimurium TA 98 shows that the nomo-NO_2 PAHs make relatively minor contributions to the mutagenicity of the urban air particles (<2%). 1-Nitropyrene was present at the highest concentration (107-1590 ppm) in the diesel particle extracts, and yet it accounted for only 3-13% of the mutagenicity. Dinitropyrene isomers present at less than 1 ppm accounted for over 25% of the mutagenicity. In urban air particle extracts NO_2-PAHs where quantified at less than 1 ppm and accounted for less than 2% of the bacterial mutagenicity. The concentration of several of the NO_2-PAHs in a series of extracts of diesel and gasoline exhaust particles were highly correlated with their mutagenicity both in S. typhimurium and L5178Y mouse lymphoma cells and skin tumor initiating activity in Sencar mice.

Bioassay-directed fractionation studies have shown that over 80% of the mutagenic activity of the solvent extractable organic material from automotive particulate emissions and ambient urban particulate is found in a compound class fraction that is more polar than PAHs.[2] This polar organic fraction is mutagenic in bacteria without the addition of an exogenous metabolic activation system, which is required to detect the

mutagenicity of unsubstituted PAHs [e.g., benzo(a)pyrene]. Studies using nitroreductase-deficient and nitroreductase-proficient strains of Salmonella typhimurium suggest that nitro-substituted compounds (e.g., NO_2-PAHs) are present in organic extracts of diesel particles and ambient air particles.[3] Many nitroarenes including nitropyrenes and other NO_2-PAHs are mutagenic in the Ames Salmonella typhimurium mutagenicity assay in the absence of exogenous metabolic activation, and are mutagenic in mammalian cells and carcinogenic in rodents.[4]

NO_2-PAHs have recently been qualitatively identified in extracts of diesel particles and urban air particulate matter.[5] However, the detection of these compounds at low levels and the quantification of NO_2-PAHs has posed problems for analytical chemists because the conventional analytical techniques for quantifying PAHs [e.g., high performance liquid chromatography (HPLC) with fluorescence detection and gas chromatography (GC)/mass spectrometry (MS) with electron impact ionization (EI)] are relatively insensitive to these nitro-substituted PAHs. The 1,3-; 1,6-; and 1,8-dinitropyrene isomers are so highly mutagenic in the Ames (TA98 -S9) assay that trace concentrations of these compounds, if present in diesel particulate extracts, could account for a major proportion of the observed mutagenic activity. A capillary column GC/MS analytical technique using on column injection and negative chemical ionization (NCI) detection was therefore developed to detect these and other nitro-PAHs at very low concentrations. The application of this technique to extracts of particles from four diesel vehicles, one gasoline catalyst vehicle and two urban air locations is reported here (Table 1). Twenty-three different NO_2-PAHs were identified in the diesel engine extracts

five NO_2-PAHs in the urban air particle extracts, and only one, 1-nitropyrene was detected in the unleaded gasoline engine extract. 1-Nitropyrene was the NO_2-PAH detected in greatest quantity in the diesel extracts (107-1590 ppm, relative to the weight of the extract), followed by the nitrophenanthrene/anthracene isomers. The quantities of these detected nitro-aromatics, which varied from 0.1 ppm to 1590 ppm in the extracts are given in Table 1. Mono-nitro derivatives of the PAHs at molecular weight 228, such as benz(a)anthracene and chrysene, and molecular weight 252, such as benzo(a)pyrene, perylene and benzofluoranthenes, were also detected. Two nitropyrenone isomers were identified in diesel samples A, B, and D. The only dinitro-PAHs for which analytical standards were available, the dinitropyrene isomers, were detected in one diesel extract sample at sub-ppm concentrations (0.4-0.6 ppm).

The quantification of NO_2-PAHs in the particle extracts reported here allows estimation of the contributions that these quantified nitro-PAHs make to the mutagenicity. Although this may be done by using the mutagenicity values reported in the literature for individual NO_2-PAH, this approach is limited by the wide variation in mutagenic activity reported for many of these compounds apparently due to both the purity of the compounds and the variations in the metabolic state of the bacterial culture. In order to minimize these errors, the five automobile emission samples described here and the pure (>99%) nitro-PAH isomers (1-nitropyrene and 3-nitrofluoranthene) were assayed in the Ames S. typhimurium plate incorporation assay simultaneously with the same resting bacterial culture of tester strain TA98 (-S9). These automotive particle emission extracts and urban air particle extracts are mutagenic in S. typhimurium TA1538,

TA1537, TA98, and TA100 and are non-mutagenic in TA1535.[3] Tester strain TA98 has been the most widely used to compare the mutagenicity of both automotive emissions and ambient air particulate matter and to compare the mutagenicity of different NO_2-PAHs and was therefore selected for this study. The calculated contribution that 1-nitropyrene, 3-nitrofluoranthene, and dinitropyrene isomers make to the "direct-acting" mutagenic activity (-S9) of the five particle extracts is shown in Table 2. Although 1-nitropyrene was present in the diesel extracts at concentrations ranging from 107 ppm to 1590 ppm (Table 1), this compound accounted for only 3% to 13% of the "direct-acting" (S-9) mutagenicity; 3-nitrofluoranthene present at 1 ppm to 7 ppm in the diesel samples accounted for 0.8% to 1.4% of the mutagenicity. By using the mutagenicity values determined in separate experiments for 2-nitrofluorene and those reported in the literature for 1-nitroaphthalene, these compounds were estimated to account for less than 0.01% of the mutagenic activity. Although the dinitropyrene isomers: 1,3; 1,6; and 1,8 were detected only in diesel sample C at 0.4-0.6 ppm, their mutagenic activity (496,000; 629,000; and 870,000 rev/μg, respectively)[6] was high enough to account for 26% of the mutagenicity of this sample. The total "direct-acting" mutagenic activity in S. typhimurium TA98 that can be accounted for by the 23 nitro-PAHs quantified in Diesel C is 40%. This estimation is supported by the loss of 50% of the mutagenic activity of this extract it was assayed in TA98FRI (renamed TA98NRD), a classical nitroreductase-deficient S. typhimurium tester strain obtained from H. Rosenkranz.[7]

The fact that dinitropyrenes at concentrations below the ppm level can account for nearly one-third of the mutagenic activity (-S9) suggests

that the presence of other highly potent dinitro-PAHs may account for even more of the mutagenic activity (-S9) in the moderately polar neutral fraction. A recent application of S. typhimurium tester strains developed to exhibit resistance to dinitropyrenes (e.g., TA98/1,8DNP$_6$) has led to even larger estimations of the contributions of dinitropyrenes to the mutagenicity of diesel particle extracts.[10] The lack of quantitative data on the concentrations of the dinitropyrene isomers in such samples has previously made it impossible to confirm whether the concentrations of the dinitropyrenes are indeed sufficient to account for the contribution predicted by the resistant tester strains. Pederson[11] has reported that in several light-duty diesel particle extracts, 50% to 90% of the TA98 (-S9) mutagenicity is lost when the extracts are tested in TA98/1,8DNP$_6$. Since these strains may show a resistance to other dinitro-PAHs, it is possible that highly potent dinitro-substituted isomers of other parent PAHs present in the particle extracts may also contribute to the mutagenicity of the moderately polar neutral fraction.

The mutagenicity of the extractable organic material from these diesel and gasoline emissions has been reported for a battery of in vitro bioassays detecting gene mutations, DNA damage, chromosomal effects, and oncogenic transformations.[12] Skin tumor initiation and carcinogenic activity of these samples has also been reported in SENCAR mice.[13] Very good correlations (r^2>0.90) were observed when the slope of the dose-response for the mutagenic activity in S. typhimurium strain TA98(-MA) was plotted versus the mutagenic activity in the mammalian cell assays and the skin tumor initiating activity.

The correlation of mutagenic and skin tumor initiating activity with the concentration of selected nitro-PAHs was examined for these diesel and gasoline samples. Table 3 shows the high correlations observed between the concentrations of 1-nitropyrene, 3-nitrofluoranthene, and several other nitro-PAH isomers in these samples, and their mutagenic activity in S. typhimurium, L5178Y mouse lymphoma cells, and skin tumor initiating activity in SENCAR mice. The r^2 correlation coefficient for the relationship between the mutagenicity in S. typhimurium (-S9) and the 3-nitrofluoranthene concentration in the series of diesel and gasoline samples is particularly good, as shown in Figure 1.

The mutagenicity in S. typhimurium (-S9) correlated well ($r^2 \geq 0.90$) with the concentrations of 3-nitrofluoranthene, the nitro-252 isomer, 1-nitropyrene, and the nitro-228 isomers, nitromethylpyrenes and nitrofluorenes while no correlation was observed with several of the other less mutagenic, lower molecular weight nitro-PAHs (nitrophenanthrenes and nitronaphthalenes). The mutagenic activity in the absence of S9 activation in L5178Y mouse lymphoma cella at the thymidine kinase locus also correlated well with the concentrations of 3-nitrofluoranthene ($r^2=.99$) and somewhat less well with 1-nitropyrene ($r^2=0.89$), and these correlations decreased with the addition of S9. The skin tumor initiating activity[28] was also highly correlated with the concentration of certain nitro-PAHs (e.g., 3-nitrofluoranthene and nitrofluorenes) as shown in Table 3.

The quantified mono-NO_2-PAHs account for less than 20% of the direct-acting bacterial mutagenicity of these samples. The high correlations observed between the concentration of these compounds and the mutagenic

activity of the total extract, therefore, suggest that the concentrations of the unidentified mutagens responsible for the remainder of the mutagenic activity and possibly the mutagenic and carcinogenic activity in other bioassays is directly related to the relative concentrations of these mono-NO_2-PAHs. Because the remaining unidentified mutagens appear to be primarily located in the chemical fractions that are more polar than the fraction which contains the unsubstituted and mono-NO_2-PAHs, it is possible that other di-NO_2-PAHs (e.g., dinitrofluoranthenes) or other oxygenated NO_2-PAH's (e.g., hydroxy-nitro-PAHs) species are primarily responsible for the major portion of the unidentified mutagenic activity.

We have recently identified and quantifed hydroxy-NO_2-PAHs in both a polar neutral fraction and acidic fraction of an ambient air particulate extract using bioassay-directed fractionation.

DISCLAIMER

The research described in this paper has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

REFERENCES

1. Huisingh, J., Bradow, R., Jungers, R., Claxton, L., Zweidinger, R., Tejada, S. Bumgarner, J., Duffield, F., Waters, M., Simmon, V.F., Hare, C., Rodriguiz, C. & Snow, L. in Application of Short-Term Bioassays in the Fractionation and Analysis of Complex Environmental Mixtures (eds. Waters, M. D., Nesnow, S., Huisingh, J. L., Sandhu, S. S. & Claxton, L.) 381-418 (Plenum Press, New York, 1979).
2. Wei, E. T., Wang, Y. Y. & Rappaport, S. M. APCA J. 30, 267-271 (1980).
3. Lewtas, J. Environmental Health Perspectives 47, 141-152 (1983).
4. Nesnow, S., Triplett, L. L. & Slaga, T. J. J. Natl. Cancer Inst. 68, 829-834 (1982).
5. Lewtas, Huisingh, J. Bull. NY Acad. Med. 57, 251-160 (1981).
6. Claxton, L. D. & Huisingh, J. D. in Pulmonary Toxicology of Respirable Particles Dept. of Energy Symposium Series 53, CONF-791002, 453-465 (U.S. Government Printing Office, Washington, D.C., 1980).
7. Loforth, G. in Short-Term Bioassays in the Analysis of Complex Mixtures II (eds. Waters, M. D., Sandhu, S. S., Lewtas Huisingh, J., Claxton, L., and Nesnow, S.) 319-336 (Plenum Press, New York, 1981).
8. Mermelstein, R., Kiriazedes, D. K., Butler, M., McCoy, E. C. & Rosenkranz, H. S. Mutat. Res. 89, 187-196 (1981).
9. Arlett, C. F., Cole, J., Broughton, B. C., Lowe, J. & Bridges, B. A. in Genotoxic Effects of Airborne Agents (eds. Tice, R. R., Costa, D. L. & Schaich, K. M.) 397-410 (Plenum, New York, 1981).
10. Sugimura, T. & Takayama, S., Environmental Health Perspectives 47, 171-176 (1983).

11. Ohgaki, H., Motsukura, N., Morino, K., Kawachi, T., Sugimura, T., Morita, K., Tokiwa, H. & Hirota, T. Cancer Lett. 15, 1-7 (1982).

12. Petersen, B. A., Chuang, C. C., Margard, W. L., and Trayser, D. A., J. Air Pollution Control Assoc., 81, 1-15 (1981).

13. Rappaport, S. M., Wang, Y. Y., Wei, E. T., Sawyer, B. E., Watkins, B. E. & Rappoport, H. Environ. Science & Tech. 14, 1505-1509 (1980).

14. Schuetzle, D., Lee, F. S.-C., Prater, T. J. & Tejada, S. B. Int. J. Environ. Anal. Chem. 9, 93-144 (1981).

15. Lewtas, J., Bradow, R. L., Jungers, R. H., Harris, B. D., Zweidinger, R. B., Cushing, K. M., Gill, B. E. & Albert, R. E. Environ. Int. 5, 383-387 (1981).

16. Claxton, L. D. Environ. Int. 5, 389-392 (1981).

17. Rosenkranz, H. S., McCoy, E. C., Mermelstein, R. & Speck, W. T. Mutat. Res. 91, 103-105 (1981).

18. McCoy, E. C., Rosenkranz, H. S. & Mermelstein, R. Environ. Mutag. 3, 421-427 (1981).

19. Pederson, T. C. in Mobile Source Emissions: Recent Impacts and Research Needs (eds. Cooke, M. & Rondia, D.) (Reidel, Dordrecht, in the press).

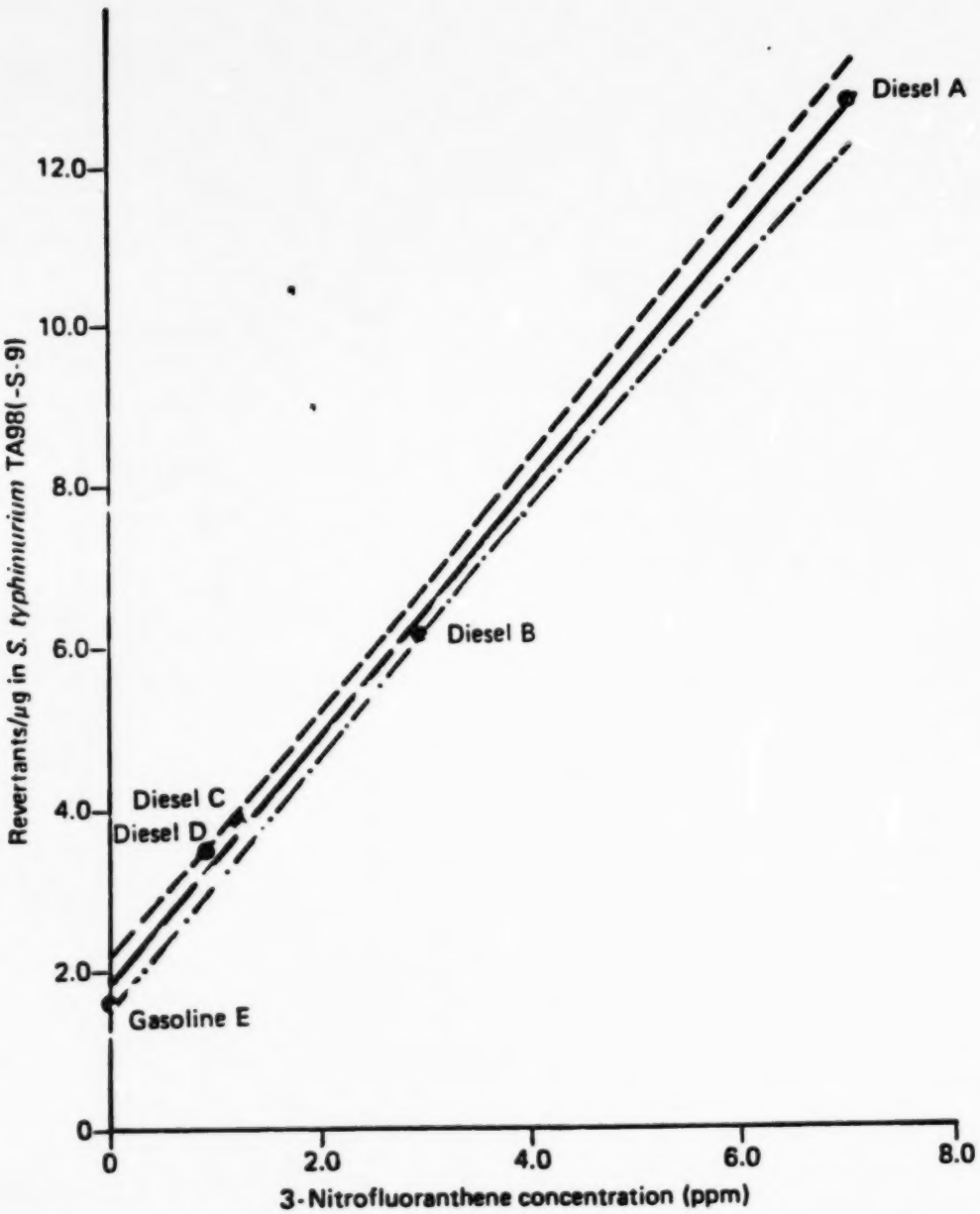

Figure 1. Correlation analysis of the concentration (PPM) of 3-nitrofluoranthene in the diesel (A-D) and gasoline (E) particulate extracts plotted versus the mutagenic activity. The mutagenic activity was determined from the linear slope of the dose-response in Salmonella typhimurium TA98 (-S9).

Table 1 Quantification of nitrated polynuclear aromatic hydrocarbons in particulate extracts

Compound	Concentration in extract (µg of species/g of extract) (ppm)						
	Diesel Auto A	Diesel Auto B	Diesel Auto C	Diesel Auto D	Gasoline Auto E	Urban Air F	Urban Air G
1-Nitronaphthalene	1.4	0.5	0.7	0.3	ND	0.1	0.2
Nitronaphthalene/azulene isomer	ND	0.1	0.6	0.2	ND	ND	ND
Nitronaphthalene/azulene isomer	1.7	0.9	1.2	0.3	ND	"	"
Nitrofluorene isomer	ND	ND	1.0	0.5	ND	"	"
2-Nitrofluorene	ND	ND	ND	0.4	ND	"	"
Nitrofluorene isomer	17.	1.9	1.4	1.1	ND	ND	ND
9-Nitroanthracene	ND	ND	ND	ND	ND	1.0	0.8
Nitrophenanthrene/anthracene isomer	272.	98.	285.	63.	ND	0.6	ND
Nitrophenanthrene/anthracene isomer	9.0	2.7	33.	25.	ND	ND	ND
3-Nitrofluoranthene	7.0	2.9	1.2	0.9	ND	ND	ND
Nitrofluoranthene/pyrene isomer	7.5	1.7	1.4	0.6	ND	0.8	0.4
1-Nitropyrene	1590.	407.	589.	107.	2.5	1.0	0.2
Nitromethylpyrene/methylfluoranthene isomer	233.	23.	46.	22.	ND	ND	ND
Nitromethylpyrene/methylfluoranthene isomer	11.	1.9	3.3	1.9	ND	"	"
Nitro-228	16.	3.0	1.1	1.4	ND	"	"
Nitro-228	7.	0.8	1.3	0.2	ND	"	"
Nitro-228	31.	3.9	5.6	1.2	ND	"	"
Nitromethyl 228	1.3	2.6	ND	ND	ND	"	"
Nitro-252	68.	29.	10.	1.1	ND	"	"
Nitropyrenone/fluoranthenone isomer	15.	5.	ND	2.1	ND	"	"
Nitropyrenone/fluoranthenone isomer	35.	12.	ND	3.9	ND	"	"
1,3-Dinitropyrene	ND	ND	0.6	ND	ND	ND	ND
1,6-Dinitropyrene	ND	ND	0.6	ND	ND	ND	ND
1,8-Dinitropyrene	ND	ND	0.4	ND	ND	ND	ND

Table 1 Quantification of nitrated polynuclear aromatic hydrocarbons in particulate extracts

Compound	Concentration in extract (µg of species/g of extract) (ppm)						
	Diesel Auto A	Diesel Auto B	Diesel Auto C	Diesel Auto D	Gasoline Auto E	Urban Air F	Urban Air G
1-Nitronaphthalene	1.4	0.5	0.7	0.3	ND	0.1	0.2
Nitronaphthalene/azulene isomer	ND	0.1	0.6	0.2	ND	ND	ND
Nitronaphthalene/azulene isomer	1.7	0.9	1.2	0.3	ND	"	"
Nitrofluorene isomer	ND	ND	1.0	0.5	ND	"	"
2-Nitrofluorene	ND	ND	ND	0.4	ND	"	"
Nitrofluorene isomer	17.	1.9	1.4	1.1	ND	ND	ND
9-Nitroanthracene	ND	ND	ND	ND	ND	1.0	0.8
Nitrophenanthrene/anthracene isomer	272.	98.	285.	63.	ND	0.6	ND
Nitrophenanthrene/anthracene isomer	9.0	2.7	33.	25.	ND	ND	ND
3-Nitrofluoranthene	7.0	2.9	1.2	0.9	ND	ND	ND
Nitrofluoranthene/pyrene isomer	7.5	1.7	1.4	0.6	ND	0.8	0.4
1-Nitropyrene	1590.	407.	589.	107.	2.5	1.0	0.2
Nitromethylpyrene/methylfluoranthene isomer	233.	23.	46.	22.	ND	ND	ND
Nitromethylpyrene/methylfluoranthene isomer	11.	1.9	3.3	1.9	ND	"	"
Nitro-228	16.	3.0	1.1	1.4	ND	"	"
Nitro-228	7.	0.8	1.3	0.2	ND	"	"
Nitro-228	31.	3.9	5.6	1.2	ND	"	"
Nitromethyl 228	1.3	2.6	ND	ND	ND	"	"
Nitro-252	68.	29.	10.	1.1	ND	"	"
Nitropyrenone/fluoranthenone isomer	15.	5.	ND	2.1	ND	"	"
Nitropyrenone/fluoranthenone isomer	35.	12.	ND	3.9	ND	"	"
1,3-Dinitropyrene	ND	ND	0.6	ND	ND	ND	ND
1,6-Dinitropyrene	ND	ND	0.6	ND	ND	ND	ND
1,8-Dinitropyrene	ND	ND	0.4	ND	ND	ND	ND

Table 1 Legend

Diesel and gasoline particulate emissions were obtained by the dilution tunnel sampling technique from the following vehicles operated on a chassis dynamometer using the U.S. highway fuel economy test cycle (HWFET):[12] a preproduction 1973 Datsun-Nissan 220 C diesel vehicle (diesel samples A and B), a prototype turbocharged Volkswagen Rabbit diesel vehicle (diesel sample C), a 1978 Oldsmobile 350 diesel vehicle (diesel sample D), and a 1977 Ford Mustang II-302 gasoline spark ignition vehicle equipped with a catalytic converter and exhaust gas recirculation emission controls (gasoline sample E). The particle emission rates for these vehicles were 0.20 g/km (diesel A and B), 0.11 g/km (diesel C), 0.32 g/km (diesel C) and 0.0033 g/km (gasoline E). The particulate emissions collected on 20" x 20" Teflon coated Pallflex T60-A20 filters, were Soxhlet extracted with dichloromethane (DCM) to remove organic material. The percent of extractable organic material for each of these samples was 11% (A), 8% (B), 18% (C), 17% (D), 43% (E) 7.6% (F) and 6.1% (G). The extracted organic material was fractionated by open bed chromatography on 5% H_2O deactivated silica gel into the following four fractions by successively more polar solvents: aliphatic fraction (hexane), aromatic fraction (hexane/benzene), moderately polar fraction (DCM) and highly polar fraction (methanol). The aromatic fraction contained all the mononitro-PAHs of 2-4 rings and the moderately polar fraction contained mononitro-PAHs of greater than 4 rings and dinitro-PAHs. The highly polar neutral fraction contained substituted PAHs with more than one substitution (e.g., nitro and keto groups as in nitrofluorenones). Capillary column gas chromatography (HRGC)/mass spectrometry (MS) with chemical ionization (methane) and negative ion detection (NCI) was used to separate and identify the nitroaromatics. On-column chromatographic injection was used and found to be essential to eliminate thermal degradation during analysis. In order to quantify the NO_2PAHs, standard solutions containing the following compounds were analyzed over the concentration range 0.1-10 ng/µl for the calibration curve: 1-nitroaphthalene, 2-nitrofluorene, 9-nitroanthracene, 1-nitropyrene, 2,7-dinitrofluorene, 2,7-dinitrofluorenone, 1,6-dinitropyrene, and 1,3,6-trinitropyrene. D_7-nitronaphthalene, the internal standard, was spiked into all standards and samples at the level of 2.5 ng/µl. Additional standards of 3-nitrofluoranthene, 1,3-dinitropyrene, and 1,8-dinitropyrene were analyzed to determine the retention times of these compounds. The response factor for each compound relative to the internal standard was calculated over the calibration range. Nitro-aromatic compounds not present in the standard that were detected in samples were quantified by using the response factor of the most similar compound from the standard. The detection limit for all the NO_2-PAHs was 0.1 µg/g of extract (ppm)±10%. The compounds not detected are shown in the table as ND. The relative retention time (relative to d_7-nitronaphthalene) and mass spectrum of each standard compound was used to assign specific isomer identification. Nitro-PAH isomers of MW 228 [e.g., benz(a)anthracene, chrysene, benz(c)phenanthrene, etc.] and MW 252 [e.g., B(e)P, B(a)P, perylene, benzofluoranthe etc.] and other isomers detected for which standards were not available were identified on the basis of their mass spectra and GC retention.

Table 2 Contribution of NO_2-PAHs to the mutagenic activity of particle extracts in Salmonella typhimurium TA98(-S9)

Extract Sample	Mutagenic Activity (rev/µg)	Contribution to Mutagenic Activity (%)		
		1-nitropyrene	3-nitrofluoranthene	dinitropyrene isomers
Diesel Auto A	13.	11.	1.4	--
Diesel Auto B	6.2	5.9	1.2	--
Diesel Auto C	3.9	13.	0.8	26.
Diesel Auto D	3.5	2.7	0.8	--
Gasoline Auto E	1.6	0.1	--	--
Urban Air F	1.8	0.05	1.8	--
Urban Air G	4.4	0.004	0.4	--

Legend

The Ames S. typhimurium TA98 plate incorporation assay was performed as described by Claxton.[13] The diesel and gasoline extracts were bioassayed simultaneously with 1-nitropyrene (1-NP) and 3-nitrofluoranthene (3-NF) (both prepared by Midwest Research Institute to greater than 99.95% purity). The diesel and gasoline extracts were tested at the following 6 doses (10, 30, 50, 100, 300, 500 µg/plate) in triplicate plates in the absence of S9. The pure nitro-PAHs, 1-NP and 3-NF were tested at 7 doses (0.020-1.00 µg/plate and 0.010-0.060 µg/plate respectively) in triplicate plates in the absence of S9. 2-Nitrofluorene (3µg) was used as a positive control and the spontaneous plate counts were 15 ± 2 rev/plate. The initial slope of the dose response curve was determined using linear regression analysis and was 892 rev/µg for 1-nitropyrene and 25,200 for 3-nitrofluoranthene. The contribution of 1-NP, 3-NF and the dinitropyrene isomers to the mutagenic activity was calculated as follows: (concentration of nitro-PAH in µg/g extract) x (mutagenicity of the nitro-PAH in rev/µg) ÷ (mutagenicity of the extract in rev/g). The mutagenicity of the dinitropyrene isomers used in these calculations was 496,000 rev/µg (1,6-dinitropyrene) and 870,000 rev/µg (1,8-dinitropyrene). The contribution of the 1-nitronapthalene and 9-nitroanthracene to the mutagenicity of the urban air particles was <0.001%.

Table 3 Correlation coefficients (r^2) for NO_2-PAHs in automotive emissions vs. mutagenic and tumorigenic activity

Nitro-PAH	*S. typhimurium* -S9	*S. typhimurium* +S9	Mouse Lymphoma -S9	Mouse Lymphoma +S9	Tumor Initiation SENCAR
1-nitropyrene	0.91	0.86	0.89	0.69	0.82
3-nitrofluoranthene	1.00	0.92	0.99	0.88	0.95
Nitro-252	0.98	0.96	0.96	0.86	0.89
Nitro-228 isomers (3)	0.93	0.95	0.97	0.87	0.91
Nitromethylpyrene isomers (2)	0.90	0.93	0.98	0.85	0.93
Nitrophenanthrene/anthracene isomers (2)	0.33	0.22	0.31	0.08	0.28
Nitronaphthalene/azulene isomers (3)	0.63	0.46	0.57	0.28	0.53
Nitrofluorene isomers (3)	0.91	0.93	0.99	0.90	0.95

Legend

The correlation coefficients (r^2) were determined by linear regression analysis of the NO_2-PAH concentrations from the diesel and gasoline automotive emissions in Table 1 versus the mutagenic and tumorigenic activity as shown in Fig. 1. Mutagenic activity in *S. typhimurium* TA98 was determined from linear regression analysis of the initial slope (revertants/μg) as shown in Table 3 in the absence of an S9 metabolic activation system (-S9). The *S. typhimurium* activity in the presence of S9 (+S9) in rev/μg was 13 (A), 3 (C), 1.5 (D), and 3.5 (E) as previously reported.[2] Mutagenic activity in L5178Y mouse lymphoma cells at the thymidine kinase locus as determined from linear regression analysis (mutation frequency/10^6 cells/μg/ml) (2) for -S9 and +S9 respectively was 4.2, 2.9 (A); 0.98, 0.72 (C); 1.2, 1.3 (D); 0.38, 1.1 (E). Tumor initiating activity in SENCAR mice (papillomas/mouse/mg) (3) was 0.59 (A), 0.24 (C), 0.31 (D), 0.17 (E). See Table 1 for an explanation of the nitro-PAH designations (e.g., nitro-252). Where more than one isomer was quantified [e.g., nitro-228 isomers (3)], all the isomers were summed (number shown in parenthesis) and the sum used in the linear regression analysis to determine the r^2.